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December 21, 2001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re/ Application of Vogels, R. et al.
Application No. As yet unassigned
Filed Herewith

Anticipated Examiner: J. Ketter
Anticipated Art Unit: 1636

HIGH THROUGHPUT SCREENING OF
GENE FUNCTION USING ADENOVIRAL
LIBRARIES FOR FUNCTIONAL GENOMICS APPLICATIONS

Attorney Docket No. 25,290-A US1

CERTIFICATE OF MAILING

I hereby certify that this document is being deposited with the United States Postal Service as Express Mail Post Office to Addressee, Mailing Label No. EL875291670US, in an envelope addressed to: Commissioner for Patents, BOX PATENT APPLICATION, Washington, DC 20231 on December 21, 2001.

Ryan White

Ryan P. White

Commissioner for Patents
Box Patent Application
Washington, DC 20231

PRELIMINARY AMENDMENT

Sir:

Prior to commencing examination of the above-identified application, please amend the application as follows.

In the Specification

Please amend the paragraph which appears at page 2, lines 4 to 6, as to read as follows.

Cross-reference to Related Application: This application is a division of U.S. Application No. 09/358,036, filed on July 21, 1999, which is a continuation-in-part of U.S. Patent application Serial No. 09/097,239, filed on June 12, 1998, the contents of the entirety of both applications are hereby incorporated by reference.

Please amend the paragraph which appears on page 7, lines 15 to 18, of the specification to read:

FIG.5: Construction of pIG.NEO. pIG.NEO contains the Neo^R operatively linked to the E1B promoter. pIG.NEO was constructed by ligating the *HpaI-ScaI* fragment of pAT.PCR2.NEO.p(A) or pIG.E1A.NEO which contains the E1B promoter and Neo^R into the *EcoRV-ScaI* sites of pBS.

Please amend the paragraph that appears on page 13, lines 17 to 18, of the specification to read:

FIG. 36 (A and B) (Example 20): Schematic overview of constructing an arrayed adenoviral cDNA expression library.

Please amend the paragraph that appears on page 13, lines 19 to 24, of the specification to read:

FIG. 37 (A, B, C, and D) (Example 21): Comparison of cotransfections of different adapter plasmids and pWE/Ad.AflII-rITRDE2A on 384-well plates with cotransfections on 96-well plates. The percentage of virus producing wells (CPE positive wells) scored at different time points as indicated after propagation of the freeze/thawed transfected cells to new PER.C6/E2A cells 5 days after transfection (upper panel) or 7 days after transfection (lower panel) is shown.

Please amend the paragraph that bridges page 13, lines 30 to page 14, line 4, of the specification to read:

FIG. 39 (A, B, and C) (Example 23): The percentage of virus producing cells (CPE positive) scored after propagation of the freeze/thawed transfected cells to new PER.C6/E2A cells, in three different experiments using PER.C6/E2A cells for transfections with indicated confluency at time of transfection. The figure legend refers to Table 9 where the absolute cell numbers from each flask in each experiment were counted. The cells from these flasks were used to seed 96-well plates for transfection with adenoviral adapter and helper DNA molecules.

Please amend the paragraph that bridges page 40, line 31, to page 41, line 5, of the specification to read:

This construct is of use when established cells are transfected with E1A.E1B constructs and NEO selection is required. Because NEO expression is directed by the E1B promoter, NEO resistant cells are expected to co-express E1A, which also is advantageous for maintaining high levels of expression of E1A during long-term culture of the cells. pIG.NEO was generated by cloning the *HpaI-ScaI* fragment of pAT.PCR2.NEO.p(A) or pIG.E1A.NEO, containing the NEO gene under the control of the Ad5 E1B promoter into pBS digested with *EcoRV* and *ScaI*.

Please amend the paragraph which bridges page 99, line 28 to page 100, line 3, of the specification to read:

In FIG.36A and FIG. 36B, an overview is given of the scheme of an adenoviral cDNA expression library constructed and arrayed as described above. This scheme describes the construction of libraries of individually cloned adenoviral vector libraries in a high throughput fashion. The improvement of this strategy over pooled libraries is that no bias for viruses with a growth advantage can occur. This is because individual members of the library are in the format of individual colonies straight after the plating of the library, and are kept individually during all further procedures.

Please amend the paragraph that bridges page 100, line 24 to page 101, line 12 of the specification to read:

Essentially, this experiment was performed as described in Example 10, except for the following minor changes. The day before transfection, PER.C6/E2A cells were diluted with culture medium (DMEM with 10% fetal bovine serum and 10 mM $MgCl_2$) to a suspension of 11,250 cells per 25 μl, followed by seeding 25 μl per well of a 384-well tissue culture plate using a 16 channel multichannel pipette (Finn). After adding 1.3 ml serum free DMEM to the DNA/lipofectamine mixture, 15 μl of this mixture was then added to each PER.C6/E2A seeded well that had been washed with 25 μl DMEM prior to transfection. After 3 hours in a humidified CO_2 incubator (39°C, 10% CO_2), 50 μl culture medium was added to each well and the plates were returned to the humidified CO_2 incubator (39°C, 10% CO_2). The next day, the medium of each well was replaced with 50 μl culture medium. The plates were then returned to a humidified CO_2 incubator (32°C, 10% CO_2) for an additional 4 days, after which the wells were subjected to freezing at -20°C overnight followed by thawing and resuspension by repeated pipetting. An aliquot of 25 μl of the freeze/thawed transfected cells was transferred to each well of a plate with fresh PER.C6/E2A cells seeded as described above on 384-well tissue culture plates (plate 2). The second 384-well plate, with PER.C6/E2A cells incubated and thus infected with freeze/thawed cell lysate of the first transfected plate, was checked for CPE formation and stored at -20°C. The experiment mentioned above was performed twice. In FIG. 37A, FIG. 37B, FIG. 37C, and FIG. 37D, the percentage of CPE positive wells scored after propagation of the freeze/thawed transfected cells to new PER.C6/E2A cells is depicted.

Please amend the paragraph which appears at page 104, lines 3 to 16, in the specification to read:

The second plate of the two transfected plates was used for virus production. Seven days after transfection, the wells of the second plate were subjected to freezing at -20°C overnight, followed by thawing and resuspension by repeated pipetting. A 100 μl aliquot of the freeze/thawed transfected cells was transferred to each well of a plate with new PER.C6/E2A cells ($2.25x10^4$ cells per well in 100 μl) that were seeded in 96-well tissue

culture plates one day prior to infections. The plate was incubated in the humidified CO_2 incubator (32°, 10% CO_2) until the presence of full CPE was observed. In FIG. 39A, FIG. 39B, and FIG. 39C, the percentage of virus producing cells (CPE positive wells), scored after propagation of the freeze/thawed transfected cells to new PER.C6/E2A cells, is depicted. The data indicate that the level of confluency of the PER.C6/E2A cells, prior to transfection with the adenoviral adapter and helper DNA molecules, influences the final percentage of virus producing wells. The higher confluency was the most optimal for absolute final number of wells producing virus and the speed at which the virus generation occurs.

In the Claims

Cancel Claims 1 to 14.

Amend Claim 15 to read as follows.

15. (Amended) A method for determining at least one function of at least one nucleic acid present in a library of claim 41, said method comprising:

transducing a multiplicity of cells with at least one vehicle comprising said at least one nucleic acid from said library, and

culturing said multiplicity of cells while allowing for expression of said at least one nucleic acid and determining the expressed function thereof.

Cancel Claims 16 to 40 (Claims 36 to 40 having been added in a Preliminary Amendment which accompanied the filing of parent Application No. 09/358,036 on July 21, 1999).

Add the following claims.

--41. A method for determining the function of a unique nucleic acid present in a library, said method comprising:

(a) providing a library of a multitude of unique expressible nucleic acids, said library including a multiplicity of compartments, each of said compartments consisting

essentially of one or more adenoviral vector comprising at least one unique nucleic acid of said library in an aqueous medium, wherein said adenoviral vector is capable of introducing said nucleic acid into a host cell, is capable of expressing the product of said nucleic acid in said host cell, and is deleted in a portion of the adenoviral genome necessary for replication thereof in said host cell;

(b) transducing a multiplicity of host cells with at least one adenoviral vector comprising at least one unique nucleic acid from said library;

(c) incubating said host cells to allow expression of the product of said nucleic acid; and

(d) determining the function of said nucleic acid.

42. The method of claim 41 wherein step (d) comprises observing said host cell to identify any changes in said host cell relative to a host cell that has not been transduced with an adenoviral vector comprising said nucleic acid.

43. The method of claim 41, wherein the function of the expression product of all of said unique expressible nucleic acids in said library is unknown at the time said library is first made.

44. The method of claim 41, wherein none of said compartments contain any adenoviral vector capable of replication except in a packaging cell containing said deleted portion of adenoviral genome.

45. The method of claim 41, wherein said host cell is a eucaryotic cell.

46. The method of claim 41, wherein at least one compartment comprises at least two adenoviral vectors.

47. The method of claim 41, wherein each of said compartments consists essentially of one said adenoviral vector.

48. The method of claim 44, wherein each of said compartments contains from about 0.01 x 10^{10} to about 10 x 10^{10} pfu of said adenoviral vector per ml of aqueous medium.

49. The method of claim 48, wherein each of said compartments further contains the cellular debris from packaging cell lysate.

50. The method of claim 44, wherein said adenoviral vector is a minimal vector.

51. The method of claim 50, wherein said minimal vector comprises an adenovirus encapsidation signal or a functional part, derivative and/or analogue thereof, and at least one copy of at least a functional part or a derivative of an adenoviral ITR.

52. The method of claim 44, wherein said adenoviral vector comprises adenoviral genomic sequence deleted for sequence encoding the E1-region proteins.

53. The method of claim 51 wherein said minimal vector further comprises an adeno-associated virus terminal repeat or a functional part, derivative and/or analogue thereof.

54. The method of claim 52, wherein said adenoviral vector is further deleted for sequence encoding the E2A-region proteins, or the E2B region proteins or the complete E2 region proteins.

55. The method of claim 41, wherein said adenoviral vector further comprises adenovirus genomic sequence encoding adenoviral fiber proteins from at least two serotypes of adenovirus.

56. The method according to claim 41, wherein said multiplicity of cells is divided over a multiplicity of compartments, each said compartment comprising at least one vector.

57. The method according to claim 41, further comprising selecting at least one vector having a desired function.

58. The method according to claim 41, wherein at least one of said performed steps is automated.

59. A method for obtaining an expressible nucleic acid having a desired function when expressed in a cell, said method comprising:

(a) performing the method of claim 41;

(b) determining which compartment in said library contains an adenoviral vector comprising a unique nucleic acid having said desired function ; and

(c) obtaining said vector from said compartment.

60. A method for producing a cellular library suitable for determining the function of a unique expressible nucleic acid, said library consisting of host cells arranged in a multiplicity of compartments, each said compartment consisting essentially of host cells transfected with a replication deficient adenoviral vector comprising at least one of said unique nucleic acids, comprising:

(a) transfecting (i) a packaging cell in each of a multiplicity of compartments and harboring a first portion of the adenoviral genome integrated into its genome, with an admixture of (ii) a nucleic acid delivery vehicle containing said unique nucleic acid operably linked to a promoter and further containing a second portion of the adenoviral genome, said second portion comprising at least one adenoviral ITR, and (iii) a helper nucleic acid consisting essentially of a third portion of the adenoviral genome;

wherein the sequence of said first portion of adenoviral genome does not overlap with the sequences of either the second or third portions of adenoviral genome; and

wherein the first, second and third portions of adenoviral genome are arranged such that all adenoviral proteins essential for replication and encapsidation are capable of expression in said packaging cells;

(b) incubating said packaging cell in each of said multiplicity of compartments under conditions which result in the lysis of said packaging cell and the release of adenoviral vectors containing said nucleic acid into each of said compartments; and

(c) transferring an aliquot of said adenoviral vectors from each of said multiplicity of compartments into a corresponding multiplicity of compartments containing said host cells.

61. The method of claim 60, wherein said sequences of said second and third portions of adenoviral genome at least partially overlap allow for homologous recombination between said delivery vehicle nucleic acid and said helper nucleic acid.

62. The method of claim 61, wherein the sequences of adenoviral genome contained in said vehicle nucleic acid, and said helper nucleic acid, do not contain any portion of the E1 region.

63. The method of claim 62, wherein said packaging cell comprises a eucaryotic cell in which genome the E1 region of the adenoviral genome is integrated.

64. The method of claim 63, wherein the sequences of said delivery vehicle and said helper nucleic acid are selected such that each is capable of being linearized by restriction enzymes that may be admixed therewith in the absence of further enzymatic restriction prior to transfection into said packaging cell.

65. A method for amplifying at least one nucleic acid present in a library consisting of a multitude of unique expressible nucleic acids arranged in a multiplicity of compartments, each said compartment consisting essentially of replication deficient adenoviral vector comprising at least one of said unique nucleic acids , comprising:

(a) transfecting (i) a packaging cell harboring a first portion of the adenoviral genome integrated into its genome, with an admixture of (ii) a nucleic acid delivery vehicle containing said unique nucleic acid operably linked to a promoter and further containing a second portion

of the adenoviral genome, said second portion comprising at least one adenoviral ITR, and (iii) a helper nucleic acid consisting essentially of a third portion of the adenoviral genome;

wherein the sequence of said first portion of adenoviral genome does not overlap with the sequences of either the second or third portions of adenoviral genome; and

wherein the first, second and third portions of adenoviral genome are arranged such that all adenoviral proteins essential for replication and encapsidation are capable of expression in said packaging cells;

(b) incubating said packaging cell under conditions which result in the lysis of said packaging cells thereby forming a cell lysate;; and

(c) introducing additional packaging cells into each compartment containing said cell lysate, and repeating steps (a) and (b) above.

66. The method according to claim 65, wherein said cell comprises said at least one nucleic acid encoding an adenoviral E1-region protein.

67. The method according to claim 66, wherein said cell is a PER.C6 cell (ECACC deposit number 96022940) or a functional derivative and/or analogue thereof.

68. The method according to claim 66, wherein said cell further comprises said at least one nucleic acid encoding adenoviral E2A and/or adenoviral E4-region protein or a functional part, derivative and/or analogue thereof.

69. The method according to claim 66, wherein said vector nucleic acid has no sequence overlap with other nucleic acids present in said cell leading to the formation of said vector nucleic acid capable of replicating in the absence of E1-region encoded proteins.

70. The method according to claim 41, wherein said multiplicity of compartments comprises a multiwell format of at least 6 wells.

71. The method according to claim 70, wherein substantially each said well consists essentially of one or more said adenoviral vector comprising said unique nucleic acid that encodes a product of unknown function.

72. The method according to claim 70, wherein said library is configured to be made and used in a substantially automated process.

73. The method according to claim 70, wherein said multiplicity of compartments comprises a multiwell format of at least 96 wells.

74. The method according to claim 73, wherein each well contains cellular debris from eucaryotic packaging cell lysate.

75. The method of claim 74, wherein none of said wells contains adenoviral vector capable of replication except in a packaging cell containing said deleted portion of adenoviral genome.

76. The method of claim 75, wherein each of said wells contains from about 0.01 x 10^{10} to about 10 x 10^{10} pfu of said adenoviral vector per ml of aqueous medium.

77. The method of claim 41, wherein each of said unique nucleic acids is derived from a member of a population of nucleic acids, said population selected from the group consisting of naturally occurring populations of messenger RNA, DNAs, cDNAs, genes, ESTs, or genetic suppressor elements, and synthetic oligonucleotides, and antisense nucleic acids.

78. The method of claim 49, wherein the contents of each said compartment is capable of transfecting said host cell and expressing the product of each said unique nucleic acid in said host cell.

79. The method of claim 78, wherein each said compartment is capable of providing from about 400 to about 4000 aliquots of said adenoviral vector.

80. The method of claim 76, wherein the contents of each said well is capable of transfecting said host cell and expressing the product of each said unique nucleic acid in said host cell.

81. The method of claim 80, wherein each said well is capable of providing from about 400 to about 4000 aliquots of said adenoviral vector.

82. The method of claim 51, wherein said minimal vector comprises an regulatable promoter operably linked to said unique nucleic acid.

83. The method of claim 52, wherein said adenoviral vector comprises an regulatable promoter operably linked to said unique nucleic acid.

84. The method of claim 52, wherein said adenoviral vector is further deleted for the adenoviral E3-region or a functional part thereof.

85. The method of claim 54, wherein said adenoviral vector is further deleted for the adenoviral E3-region or a functional part thereof.

86. The method of claim 84, wherein said adenoviral vector is further deleted for the adenoviral E4-region or a functional part thereof.

87. The method of claim 85, wherein said adenoviral vector is further deleted for the adenoviral E4-region or a functional part thereof.

88. The method of claim 82, wherein said promoter is repressed by an adenoviral E1 gene product.

89. The method of claim 83, wherein said promoter is repressed by an adenoviral E1 gene product.

90. The method of claim 88, wherein said promoter is an AP1 dependent promoter.

91. The method of claim 89, wherein said promoter is an AP1 dependent promoter.

92. A method for producing a library of claim 63, wherein said delivery vehicle comprises one or more restriction site sequence capable of cleavage by an enzyme that does not digest sequences coded for by said unique nucleic acids.

93. A method for producing a library of claim 92, wherein said restriction site sequence comprises a recognition sequence for a rare-cutting restriction endonuclease or an intron-encoded endonuclease.

94. A method according to claim 41, wherein said adenoviral vector is packaged into an adenoviral capsid.

95. A method according to claim 60, wherein said packaging cells are PER.C6 cells or derived from PER.C6 cells.

96. A method according to claim 95, wherein said cells include adenoviral genome sequence of the E2 region.

97. The method of claim 41 wherein said function comprises a biological activity.

98. The method of claim 97 wherein said biological activity is selected from the group consisting of altered viability, morphologic changes, apoptosis, DNA synthesis, tumorigenesis, disease or drug susceptibility, chemical responsiveness, chemical secretion and protein expression.

99. The method of claim 41 wherein said unique expressible nucleic acid is derived from the group consisting of mammals, fish, nemotodes, insects, yeasts, fungi, bacteria and plants.

100. The method of claim 99 wherein said library of unique nucleic acid is derived from human placenta mRNA.

101. The method of claim 99 wherein said library of unique nucleic acid is derived from zebrafish mRNAs.

102. The method of claim 99 wherein said host cells are present in zebrafish embryos.

103. The method of claim 99 wherein said host cells are present in zebrafish adults.

104. A method for producing a library according to claim 60 wherein polyethylenimine (PEI) is used as a transfection reagent to transfect said packaging cell.

105. A library of a multitude of unique expressible nucleic acids derived from placental mRNAs, said library including a multiplicity of compartments, each of said compartments consisting essentially of one or more adenoviral vector comprising at least one unique nucleic acid isolated from placental mRNA in an aqueous medium, wherein said adenoviral vector is capable of introducing said nucleic acid into a host cell, is capable of expressing the product of said nucleic acid in said host cell, and is deleted in a portion of the adenoviral genome necessary for replication thereof in said host cell.

106. A method for producing a zebrafish cellular library suitable for determining the function of a unique expressible nucleic acid, said library consisting of a multitude of zebrafish host cells in which are expressed unique expressible nucleic acids arranged in a multiplicity of compartments, each said compartment consisting essentially zebrafish host cells

transfected with a replication deficient adenoviral vector comprising at least one of said unique nucleic acids, comprising:

(a) transfecting (i) packaging cells each in a compartment of a multiplicity of compartments and harboring a first portion of the adenoviral genome integrated into its genome, with an admixture of (ii) a nucleic acid delivery vehicle containing said unique nucleic acid operably linked to a promoter and further containing a second portion of the adenoviral genome, said second portion comprising at least one adenoviral ITR, and (iii) a helper nucleic acid consisting essentially of a third portion of the adenoviral genome;

wherein the sequence of said first portion of adenoviral genome does not overlap with the sequences of either the second or third portions of adenoviral genome; and

wherein the first, second and third portions of adenoviral genome are arranged such that all adenoviral proteins essential for replication and encapsidation are capable of expression in said packaging cells;

(b) incubating said packaging cells in said compartments under conditions which result in the lysis of said packaging cells; and

(c) transferring an aliquot of said cell lysate from each of said multiplicity of compartments into a corresponding multiplicity of compartments containing said zebrafish host cells.

107. The method of claim 106 wherein said zebrafish host cells comprise embryos.

108. The method of claim 106 said zebrafish host cells comprise adult cells.

109. A method for determining the function of a unique nucleic acid present in a library, said method comprising:

(a) growing a plurality of cell cultures containing at least one cell, said one cell expressing adenoviral sequence consisting essentially of E1-region sequences and expressing one or more functional gene products encoded by at least one adenoviral region selected from an E2A region and an E4 region; and

(b) transfecting, under conditions whereby said recombinant adenovirus vector library is produced, said at least one cell in each of said plurality of cell cultures with

i) an adapter plasmid comprising adenoviral sequence coding, in operable configuration, for a functional Inverted Terminal Repeat, a functional encapsidation signal, and sequences sufficient to allow for homologous recombination with a first recombinant nucleic acid, and not coding for E1 region sequences which overlap with E1 region sequences in said at least one cell, for E1 region sequences which overlap with E1 region sequences in a first recombinant nucleic acid, for E2B region sequences other than essential E2B sequences, for E2A region sequences, for E3 region sequences and for E4 region sequences, and further comprises a unique nucleic acid sequence and promoter operatively linked to said unique nucleic acid sequence; and

ii) a first recombinant nucleic acid comprising adenoviral sequence coding, in operable configuration, for a functional adenoviral Inverted Terminal Repeat and for sequences sufficient for replication in said at least one cell, but not comprising adenoviral E1 region sequences which overlap with E1 sequences in said at least one cell, and not comprising E2A region sequences or E4 region sequences expressed in said plurality of cells which would otherwise lead to production of replication competent adenovirus wherein said first recombinant nucleic acid has sufficient overlap with said adapter plasmid to provide for homologous recombination resulting in production of recombinant adenoviral vectors in said at least one cell;

(c) incubating said plurality of cells under conditions which result in the lysis of said plurality of cells facilitating the release of said recombinant adenoviral vectors containing said unique nucleic acid; and

(d) transferring an aliquot of said adenoviral vectors into a corresponding plurality of host cell cultures consisting of cells in which said vectors do not replicate, but in which said nucleic acids are expressible;

(e) incubating said host cells to allow expression of the product of said nucleic acid; and

(f) observing said host cell for changes in said host cell.

110. A method according to claim 109, further comprising

(g) assigning a function to said nucleic acids, for which the expressed product thereof results in observed changes in said host cells.

111. A method according to claim 110, wherein said biological function comprises apoptosis, DNA synthesis, tumorigenesis, disease or drug susceptibility, chemical responsiveness, chemical secretion, protein expression, cell differentiation, proliferation, drug resistance, capillary formation, or cell migration.

112. A method according to claim 110, wherein said observing uses an assay selected from the group consisting of a beta-galactosidase assay, hIL3 assay, Luciferase assay, ceNOS assay, GLVR2 assay and EGFP assay.

113. A method according to claim 110 wherein said zebrafish host cells comprise embryos.

114. The method of claim 110 said zebrafish host cells comprise adult cells.

115. A library of a multitude of unique expressible nucleic acids derived from zebrafish mRNAs, said library including a multiplicity of compartments, each of said compartments consisting essentially of one or more adenoviral vector comprising at least one unique nucleic acid isolated from placental mRNA in an aqueous medium, wherein said adenoviral vector is capable of introducing said nucleic acid into a host cell, is capable of expressing the product of said nucleic acid in said host cell, and is deleted in a portion of the adenoviral genome necessary for replication thereof in said host cell.--

<u>In the Drawings</u>

Applicants are submitting herewith copies of drawings corrected in red ink under MPEP §608.02(v) and formal drawings consistent with these changes, as well as those changes and corrections specified in the Notice of Draftsperson's Patent Drawing Review dated August

20, 1999 in parent Application No. 09/358,036. These drawing changes and corrections were accepted in the parent Application No. 09/358,036 and are not new matter.

REMARKS

Applicants note that Claims 1 to 35 were presented in parent Application No. 09/358,036 as originally filed. Claims 36 to 40 were added in a Preliminary Amendment which accompanied the filing of Application No. 09/358,036 on July 21, 1999.

Below are summaries of the proposed amendments to the enclosed drawings.

Figure 1. Make "HBV poly (A) signal" region in pTN plasmid sequence more visible by changing border color to black. Support for this revision is found in the specification on page 40, lines 8 to 9, which describes the pTN plasmid sequence. Change border to black for figure legend as well.

Figure 2. Close pAT.X/S and pIG.E1a.E1b.X plasmid sequences with a black line. Plasmids pAT.X/S and pIG.E1a.E1b.X are shown in Figures 3A and 4 and Figure 3B respectively.

Figure 3A. Make "HBV poly (A) signal" region in pTN and pAT.PCR2.NEO plasmid sequences more visible by changing border colors to black. Support for this revision is found in the specification on page 40, lines 8 to 9, which describes the pTN plasmid sequence. Change border to black for figure legend as well. See revised figure. Also, the plasmid "pAT PCR2" should include "X/S." inserted into its name to become "pAT.X/S.PCR2". Support for this revision is found Page 40, lines 1 to 16 of the specification, specifically on lines 7 to 8.

Figure 3B. Make "HBV poly (A) signal" region in pTN, pAT.PCR2.NEO, pAT.PCR2.NEO.p(A), and pIG.E1A.NEO plasmid sequences more visible by changing border colors to black. Support for this revision is found in the specification on page 40, lines 8 to 9, which describes the pTN plasmid sequence. Change border to black for figure legend as well.

Figure 4. Make "HBV poly (A) signal" region in pIG.E1A.NEO and pIG.E1A.E1B plasmid sequences more visible by changing border colors to black. Support for this revision is found in the specification on page 40, lines 8 to 9, which describes the pTN plasmid sequence. Change border to black for figure legend as well.

Figure 5. Make "HBV poly (A) signal" region in pAT.PCR2.NEO.p(A) plasmid sequence more visible by changing border color to black. Support for this revision is found in the specification on page 40, lines 8 to 9, which describes the pTN plasmid sequence. Change border to black for figure legend as well.

Figure 13. Change "proved" to "provide".

All of the above amendments to the description and the drawings were submitted and approved in the parent application, U.S. Application No. 09/358,036. Accordingly, these amendments do not constitute new matter.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with Markings to Show Changes Made."

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification

The paragraph which appears at page 2, lines 4 to 6, has been amended to read as follows.

Cross-reference to Related Application: This application is a division of U.S. Application No. 09/358,036, filed on July 21, 1999, which is a continuation-in-part of U.S. Patent application Serial No. 09/097,239, filed on June 12, 1998, the contents of the entirety of both applications [which] are hereby incorporated by [this] reference.

The paragraph which appears at page 7, lines 15 to 18, of the specification has been amended as follows:

FIG.5: Construction of pIG.NEO. pIG.NEO contains the Neo^{R} operatively linked to the E1B promoter. pIG.NEO was constructed by ligating the *HpaI-ScaI* fragment of pAT.PCR2.NEO.p(A) or pIG.E1A.NEO which contains the E1B promoter and Neo^{R} into the *EcoRV-ScaI* sites of pBS.

The paragraph which appears at page 13, lines 17 to 18, of the specification has been amended as follows:

FIG. 36 (A[I] and B[II]) (Example 20): Schematic overview of constructing an arrayed adenoviral cDNA expression library.

The paragraph which appears at page 13, lines 19 to 24, of the specification has been amended as follows:

FIG. 37 (A, B, C, and D) (Example 21): Comparison of cotransfections of different adapter plasmids and pWE/Ad.AflII-rITRDE2A on 384-well plates with cotransfections on 96-well plates. The percentage of virus producing wells (CPE positive wells) scored at different time points as indicated after propagation of the freeze/thawed transfected cells to new PER.C6/E2A cells 5 days after transfection (upper panel) or 7 days after transfection (lower panel) is shown.

The paragraph which bridges page 13, line 30 to page 14, line 4, of the specification has been amended as follows:

FIG. 39 (A, B, and C) (Example 23): The percentage of virus producing cells (CPE positive) scored after propagation of the freeze/thawed transfected cells to new PER.C6/E2A cells, in three different experiments using PER.C6/E2A cells for transfections with indicated confluency at time of transfection. The figure legend refers to Table 9 where the absolute cell numbers from each flask in each experiment were counted. The cells from these flasks were used to seed 96-well plates for transfection with adenoviral adapter and helper DNA molecules.

The paragraph which bridges page 40, line 31 to page 41, line 5, of the specification has been amended as follows:

This construct is of use when established cells are transfected with E1A.E1B constructs and NEO selection is required. Because NEO expression is directed by the E1B promoter, NEO resistant cells are expected to co-express E1A, which also is advantageous for maintaining high levels of expression of E1A during long-term culture of the cells. pIG.NEO was generated by cloning the *HpaI-ScaI* fragment of pAT.PCR2.NEO.p(A) or pIG.E1A.NEO, containing the NEO gene under the control of the Ad5 E1B promoter into pBS digested with *EcoRV* and *ScaI*.

The paragraph which bridges page 99, line 28 to page 100, line 3, of the specification has been amended as follows:

In FIG.36A and FIG. 36B, an overview is given of the scheme of an adenoviral cDNA expression library constructed and arrayed as described above. This scheme describes the construction of libraries of individually cloned adenoviral vector libraries in a high throughput fashion. The improvement of this strategy over pooled libraries is that no bias for viruses with a growth advantage can occur. This is because individual members of the library are in the format of individual colonies straight after the plating of the library, and are kept individually during all further procedures.

The paragraph that bridges page 100, line 24, to page 101, line 12 of the specification has been amended as follows:

Essentially, this experiment was performed as described in Example 10, except for the following minor changes. The day before transfection, PER.C6/E2A cells were diluted with culture medium (DMEM with 10% fetal bovine serum and 10 mM $MgCl_2$) to a suspension of 11,250 cells per 25 μl, followed by seeding 25 μl per well of a 384-well tissue culture plate using a 16 channel multichannel pipette (Finn). After adding 1.3 ml serum free DMEM to the DNA/lipofectamine mixture, 15 μl of this mixture was then added to each PER.C6/E2A seeded well that had been washed with 25 μl DMEM prior to transfection. After 3 hours in a humidified CO_2 incubator (39°C, 10% CO_2), 50 μl culture medium was added to each well and the plates were returned to the humidified CO_2 incubator (39°C, 10% CO_2). The next day, the medium of each well was replaced with 50 μl culture medium. The plates were then returned to a humidified CO_2 incubator (32°C, 10% CO_2) for an additional 4 days, after which the wells were subjected to freezing at -20°C overnight followed by thawing and resuspension by repeated pipetting. An aliquot of 25 μl of the freeze/thawed transfected cells was transferred to each well of a plate with fresh PER.C6/E2A cells seeded as described above on 384-well tissue culture plates (plate 2). The second 384-well plate, with PER.C6/E2A cells incubated and thus infected with freeze/thawed cell lysate of the first transfected plate, was checked for CPE formation and stored at -20°C. The experiment mentioned above was performed twice. In FIG. 37A, FIG. 37B, FIG. 37C, and FIG. 37D, the percentage of CPE positive wells scored after propagation of the freeze/thawed transfected cells to new PER.C6/E2A cells is depicted.

The paragraph which appears at page 104, lines 3 to 16, in the specification have been amended as follows:

The second plate of the two transfected plates was used for virus production. Seven days after transfection, the wells of the second plate were subjected to freezing at -20°C overnight, followed by thawing and resuspension by repeated pipetting. A 100 μl aliquot of the freeze/thawed transfected cells was transferred to each well of a plate with new PER.C6/E2A cells (2.25×10^4 cells per well in 100 μl) that were seeded in 96-well tissue culture plates one day prior to infections. The plate was incubated in the humidified CO_2

incubator (32 °, 10% CO_2) until the presence of full CPE was observed. In FIG. 39A, FIG. 39B, and FIG. 39C, the percentage of virus producing cells (CPE positive[)] wells), scored after propagation of the freeze/thawed transfected cells to new PER.C6/E2A cells, is depicted. The data indicate that the level of confluency of the PER.C6/E2A cells, prior to transfection with the adenoviral adapter and helper DNA molecules, influences the final percentage of virus producing wells. The higher confluency was the most optimal for absolute final number of wells producing virus and the speed at which the virus generation occurs.

In the Claims

Cancel Claims 1 to 14.

Claim 15 has been amended as follows.

15. (Amended) A method for determining at least one function of at least one nucleic acid present in a library of [any one of claims 1-14] claim 41, said method comprising:

transducing a multiplicity of cells with at least one vehicle comprising said at least one nucleic acid from said library, and

culturing said multiplicity of cells while allowing for expression of said at least one nucleic acid and determining the expressed function thereof. 2 to 40 have been canceled.

Cancel Claims 16 to 40.

The following claims have been added.

--41. A method for determining the function of a unique nucleic acid present in a library, said method comprising:

(a) providing a library of a multitude of unique expressible nucleic acids, said library including a multiplicity of compartments, each of said compartments consisting essentially of one or more adenoviral vector comprising at least one unique nucleic acid of said library in an aqueous medium, wherein said adenoviral vector is capable of introducing said

nucleic acid into a host cell, is capable of expressing the product of said nucleic acid in said host cell, and is deleted in a portion of the adenoviral genome necessary for replication thereof in said host cell;

(b) transducing a multiplicity of host cells with at least one adenoviral vector comprising at least one unique nucleic acid from said library;

(c) incubating said host cells to allow expression of the product of said nucleic acid; and

(d) determining the function of said nucleic acid.

42. The method of claim 41 wherein step (d) comprises observing said host cell to identify any changes in said host cell relative to a host cell that has not been transduced with an adenoviral vector comprising said nucleic acid.

43. The method of claim 41, wherein the function of the expression product of all of said unique expressible nucleic acids in said library is unknown at the time said library is first made.

44. The method of claim 41, wherein none of said compartments contain any adenoviral vector capable of replication except in a packaging cell containing said deleted portion of adenoviral genome.

45. The method of claim 41, wherein said host cell is a eucaryotic cell.

46. The method of claim 41, wherein at least one compartment comprises at least two adenoviral vectors.

47. The method of claim 41, wherein each of said compartments consists essentially of one said adenoviral vector.

48. The method of claim 44, wherein each of said compartments contains from about 0.01 x 10^{10} to about 10 x 10^{10} pfu of said adenoviral vector per ml of aqueous medium.

49. The method of claim 48, wherein each of said compartments further contains the cellular debris from packaging cell lysate.

50. The method of claim 44, wherein said adenoviral vector is a minimal vector.

51. The method of claim 50, wherein said minimal vector comprises an adenovirus encapsidation signal or a functional part, derivative and/or analogue thereof, and at least one copy of at least a functional part or a derivative of an adenoviral ITR.

52. The method of claim 44, wherein said adenoviral vector comprises adenoviral genomic sequence deleted for sequence encoding the E1-region proteins.

53. The method of claim 51 wherein said minimal vector further comprises an adeno-associated virus terminal repeat or a functional part, derivative and/or analogue thereof.

54. The method of claim 52, wherein said adenoviral vector is further deleted for sequence encoding the E2A-region proteins, or the E2B region proteins or the complete E2 region proteins.

55. The method of claim 41, wherein said adenoviral vector further comprises adenovirus genomic sequence encoding adenoviral fiber proteins from at least two serotypes of adenovirus.

56. The method according to claim 41, wherein said multiplicity of cells is divided over a multiplicity of compartments, each said compartment comprising at least one vector.

57. The method according to claim 41, further comprising selecting at least one vector having a desired function.

58. The method according to claim 41, wherein at least one of said performed steps is automated.

59. A method for obtaining an expressible nucleic acid having a desired function when expressed in a cell, said method comprising:

(a) performing the method of claim 41;

(b) determining which compartment in said library contains an adenoviral vector comprising a unique nucleic acid having said desired function ; and

(c) obtaining said vector from said compartment.

60. A method for producing a cellular library suitable for determining the function of a unique expressible nucleic acid, said library consisting of host cells arranged in a multiplicity of compartments, each said compartment consisting essentially of host cells transfected with a replication deficient adenoviral vector comprising at least one of said unique nucleic acids, comprising:

(a) transfecting (i) a packaging cell in each of a multiplicity of compartments and harboring a first portion of the adenoviral genome integrated into its genome, with an admixture of (ii) a nucleic acid delivery vehicle containing said unique nucleic acid operably linked to a promoter and further containing a second portion of the adenoviral genome, said second portion comprising at least one adenoviral ITR, and (iii) a helper nucleic acid consisting essentially of a third portion of the adenoviral genome;

wherein the sequence of said first portion of adenoviral genome does not overlap with the sequences of either the second or third portions of adenoviral genome; and

wherein the first, second and third portions of adenoviral genome are arranged such that all adenoviral proteins essential for replication and encapsidation are capable of expression in said packaging cells;

(b) incubating said packaging cell in each of said multiplicity of compartments under conditions which result in the lysis of said packaging cell and the release of adenoviral vectors containing said nucleic acid into each of said compartments; and

(c) transferring an aliquot of said adenoviral vectors from each of said multiplicity of compartments into a corresponding multiplicity of compartments containing said host cells.

61. The method of claim 60, wherein said sequences of said second and third portions of adenoviral genome at least partially overlap allow for homologous recombination between said delivery vehicle nucleic acid and said helper nucleic acid.

62. The method of claim 61, wherein the sequences of adenoviral genome contained in said vehicle nucleic acid, and said helper nucleic acid, do not contain any portion of the E1 region.

63. The method of claim 62, wherein said packaging cell comprises a eucaryotic cell in which genome the E1 region of the adenoviral genome is integrated.

64. The method of claim 63, wherein the sequences of said delivery vehicle and said helper nucleic acid are selected such that each is capable of being linearized by restriction enzymes that may be admixed therewith in the absence of further enzymatic restriction prior to transfection into said packaging cell.

65. A method for amplifying at least one nucleic acid present in a library consisting of a multitude of unique expressible nucleic acids arranged in a multiplicity of compartments, each said compartment consisting essentially of replication deficient adenoviral vector comprising at least one of said unique nucleic acids , comprising:

(a) transfecting (i) a packaging cell harboring a first portion of the adenoviral genome integrated into its genome, with an admixture of (ii) a nucleic acid delivery vehicle containing said unique nucleic acid operably linked to a promoter and further containing a second portion of the adenoviral genome, said second portion comprising at least one adenoviral ITR, and (iii) a helper nucleic acid consisting essentially of a third portion of the adenoviral genome;

wherein the sequence of said first portion of adenoviral genome does not overlap with the sequences of either the second or third portions of adenoviral genome; and

wherein the first, second and third portions of adenoviral genome are arranged such that all adenoviral proteins essential for replication and encapsidation are capable of expression in said packaging cells;

(b) incubating said packaging cell under conditions which result in the lysis of said packaging cells thereby forming a cell lysate;; and

(c) introducing additional packaging cells into each compartment containing said cell lysate, and repeating steps (a) and (b) above.

66. The method according to claim 65, wherein said cell comprises said at least one nucleic acid encoding an adenoviral E1-region protein.

67. The method according to claim 66, wherein said cell is a PER.C6 cell (ECACC deposit number 96022940) or a functional derivative and/or analogue thereof.

68. The method according to claim 66, wherein said cell further comprises said at least one nucleic acid encoding adenoviral E2A and/or adenoviral E4-region protein or a functional part, derivative and/or analogue thereof.

69. The method according to claim 66, wherein said vector nucleic acid has no sequence overlap with other nucleic acids present in said cell leading to the formation of said vector nucleic acid capable of replicating in the absence of E1-region encoded proteins.

70. The method according to claim 41, wherein said multiplicity of compartments comprises a multiwell format of at least 6 wells.

71. The method according to claim 70, wherein substantially each said well consists essentially of one or more said adenoviral vector comprising said unique nucleic acid that encodes a product of unknown function.

72. The method according to claim 70, wherein said library is configured to be made and used in a substantially automated process.

73. The method according to claim 70, wherein said multiplicity of compartments comprises a multiwell format of at least 96 wells.

74. The method according to claim 73, wherein each well contains cellular debris from eucaryotic packaging cell lysate.

75. The method of claim 74, wherein none of said wells contains adenoviral vector capable of replication except in a packaging cell containing said deleted portion of adenoviral genome.

76. The method of claim 75, wherein each of said wells contains from about 0.01 x 10^{10} to about 10 x 10^{10} pfu of said adenoviral vector per ml of aqueous medium.

77. The method of claim 41, wherein each of said unique nucleic acids is derived from a member of a population of nucleic acids, said population selected from the group consisting of naturally occurring populations of messenger RNA, DNAs, cDNAs, genes, ESTs, or genetic suppressor elements, and synthetic oligonucleotides, and antisense nucleic acids.

78. The method of claim 49, wherein the contents of each said compartment is capable of transfecting said host cell and expressing the product of each said unique nucleic acid in said host cell.

79. The method of claim 78, wherein each said compartment is capable of providing from about 400 to about 4000 aliquots of said adenoviral vector.

80. The method of claim 76, wherein the contents of each said well is capable of transfecting said host cell and expressing the product of each said unique nucleic acid in said host cell.

81. The method of claim 80, wherein each said well is capable of providing from about 400 to about 4000 aliquots of said adenoviral vector.

82. The method of claim 51, wherein said minimal vector comprises an regulatable promoter operably linked to said unique nucleic acid.

83. The method of claim 52, wherein said adenoviral vector comprises an regulatable promoter operably linked to said unique nucleic acid.

84. The method of claim 52, wherein said adenoviral vector is further deleted for the adenoviral E3-region or a functional part thereof.

85. The method of claim 54, wherein said adenoviral vector is further deleted for the adenoviral E3-region or a functional part thereof.

86. The method of claim 84, wherein said adenoviral vector is further deleted for the adenoviral E4-region or a functional part thereof.

87. The method of claim 85, wherein said adenoviral vector is further deleted for the adenoviral E4-region or a functional part thereof.

88. The method of claim 82, wherein said promoter is repressed by an adenoviral E1 gene product.

89. The method of claim 83, wherein said promoter is repressed by an adenoviral E1 gene product.

90. The method of claim 88, wherein said promoter is an AP1 dependent promoter.

91. The method of claim 89, wherein said promoter is an AP1 dependent promoter.

92. A method for producing a library of claim 63, wherein said delivery vehicle comprises one or more restriction site sequence capable of cleavage by an enzyme that does not digest sequences coded for by said unique nucleic acids.

93. A method for producing a library of claim 92, wherein said restriction site sequence comprises a recognition sequence for a rare-cutting restriction endonuclease or an intron-encoded endonuclease.

94. A method according to claim 41, wherein said adenoviral vector is packaged into an adenoviral capsid.

95. A method according to claim 60, wherein said packaging cells are PER.C6 cells or derived from PER.C6 cells.

96. A method according to claim 95, wherein said cells include adenoviral genome sequence of the E2 region.

97. The method of claim 41 wherein said function comprises a biological activity.

98. The method of claim 97 wherein said biological activity is selected from the group consisting of altered viability, morphologic changes, apoptosis, DNA synthesis, tumorigenesis, disease or drug susceptibility, chemical responsiveness, chemical secretion and protein expression.

99. The method of claim 41 wherein said unique expressible nucleic acid is derived from the group consisting of mammals, fish, nemotodes, insects, yeasts, fungi, bacteria and plants.

100. The method of claim 99 wherein said library of unique nucleic acid is derived from human placenta mRNA.

101. The method of claim 99 wherein said library of unique nucleic acid is derived from zebrafish mRNAs.

102. The method of claim 99 wherein said host cells are present in zebrafish embryos.

103. The method of claim 99 wherein said host cells are present in zebrafish adults.

104. A method for producing a library according to claim 60 wherein polyethylenimine (PEI) is used as a transfection reagent to transfect said packaging cell.

105. A library of a multitude of unique expressible nucleic acids derived from placental mRNAs, said library including a multiplicity of compartments, each of said compartments consisting essentially of one or more adenoviral vector comprising at least one unique nucleic acid isolated from placental mRNA in an aqueous medium, wherein said adenoviral vector is capable of introducing said nucleic acid into a host cell, is capable of expressing the product of said nucleic acid in said host cell, and is deleted in a portion of the adenoviral genome necessary for replication thereof in said host cell.

106. A method for producing a zebrafish cellular library suitable for determining the function of a unique expressible nucleic acid, said library consisting of a multitude of zebrafish host cells in which are expressed unique expressible nucleic acids arranged in a multiplicity of compartments, each said compartment consisting essentially zebrafish host cells transfected with a replication deficient adenoviral vector comprising at least one of said unique nucleic acids, comprising:

(a) transfecting (i) packaging cells each in a compartment of a multiplicity of compartments and harboring a first portion of the adenoviral genome integrated into its genome, with an admixture of (ii) a nucleic acid delivery vehicle containing said unique nucleic acid operably linked to a promoter and further containing a second portion of the adenoviral genome, said second portion comprising at least one adenoviral ITR, and (iii) a helper nucleic acid consisting essentially of a third portion of the adenoviral genome;

wherein the sequence of said first portion of adenoviral genome does not overlap with the sequences of either the second or third portions of adenoviral genome; and

wherein the first, second and third portions of adenoviral genome are arranged such that all adenoviral proteins essential for replication and encapsidation are capable of expression in said packaging cells;

(b) incubating said packaging cells in said compartments under conditions which result in the lysis of said packaging cells; and

(c) transferring an aliquot of said cell lysate from each of said multiplicity of compartments into a corresponding multiplicity of compartments containing said zebrafish host cells.

107. The method of claim 106 wherein said zebrafish host cells comprise embryos.

108. The method of claim 106 said zebrafish host cells comprise adult cells.

109. A method for determining the function of a unique nucleic acid present in a library, said method comprising:

(a) growing a plurality of cell cultures containing at least one cell, said one cell expressing adenoviral sequence consisting essentially of E1-region sequences and expressing one or more functional gene products encoded by at least one adenoviral region selected from an E2A region and an E4 region; and

(b) transfecting, under conditions whereby said recombinant adenovirus vector library is produced, said at least one cell in each of said plurality of cell cultures with

i) an adapter plasmid comprising adenoviral sequence coding, in operable configuration, for a functional Inverted Terminal Repeat, a functional encapsidation signal, and sequences sufficient to allow for homologous recombination with a first recombinant nucleic acid, and not coding for E1 region sequences which overlap with E1 region sequences in said at least one cell, for E1 region sequences which overlap with E1 region sequences in a first recombinant nucleic acid, for E2B region sequences other than essential E2B sequences, for E2A region sequences, for E3 region sequences and for E4 region sequences, and further comprises a unique nucleic acid sequence and promoter operatively linked to said unique nucleic acid sequence; and

ii) a first recombinant nucleic acid comprising adenoviral sequence coding, in operable configuration, for a functional adenoviral Inverted Terminal Repeat and for sequences sufficient for replication in said at least one cell, but not comprising adenoviral E1 region sequences which overlap with E1 sequences in said at least one cell, and not comprising E2A

region sequences or E4 region sequences expressed in said plurality of cells which would otherwise lead to production of replication competent adenovirus wherein said first recombinant nucleic acid has sufficient overlap with said adapter plasmid to provide for homologous recombination resulting in production of recombinant adenoviral vectors in said at least one cell;

(c) incubating said plurality of cells under conditions which result in the lysis of said plurality of cells facilitating the release of said recombinant adenoviral vectors containing said unique nucleic acid; and

(d) transferring an aliquot of said adenoviral vectors into a corresponding plurality of host cell cultures consisting of cells in which said vectors do not replicate, but in which said nucleic acids are expressible;

(e) incubating said host cells to allow expression of the product of said nucleic acid; and

(f) observing said host cell for changes in said host cell.

110. A method according to claim 109, further comprising

(g) assigning a function to said nucleic acids, for which the expressed product thereof results in observed changes in said host cells.

111. A method according to claim 110, wherein said biological function comprises apoptosis, DNA synthesis, tumorigenesis, disease or drug susceptibility, chemical responsiveness, chemical secretion, protein expression, cell differentiation, proliferation, drug resistance, capillary formation, or cell migration.

112. A method according to claim 110, wherein said observing uses an assay selected from the group consisting of a beta-galactosidase assay, hIL3 assay, Luciferase assay, ceNOS assay, GLVR2 assay and EGFP assay.

113. A method according to claim 110 wherein said zebrafish host cells comprise embryos.

114. The method of claim 110 said zebrafish host cells comprise adult cells.

115. A library of a multitude of unique expressible nucleic acids derived from zebrafish mRNAs, said library including a multiplicity of compartments, each of said compartments consisting essentially of one or more adenoviral vector comprising at least one unique nucleic acid isolated from placental mRNA in an aqueous medium, wherein said adenoviral vector is capable of introducing said nucleic acid into a host cell, is capable of expressing the product of said nucleic acid in said host cell, and is deleted in a portion of the adenoviral genome necessary for replication thereof in said host cell.--

<u>In the Drawings</u>

Please see amended Figures 1, 2, 3A, 3B, 4, 5, and 13. Proposed changes are shown in red.

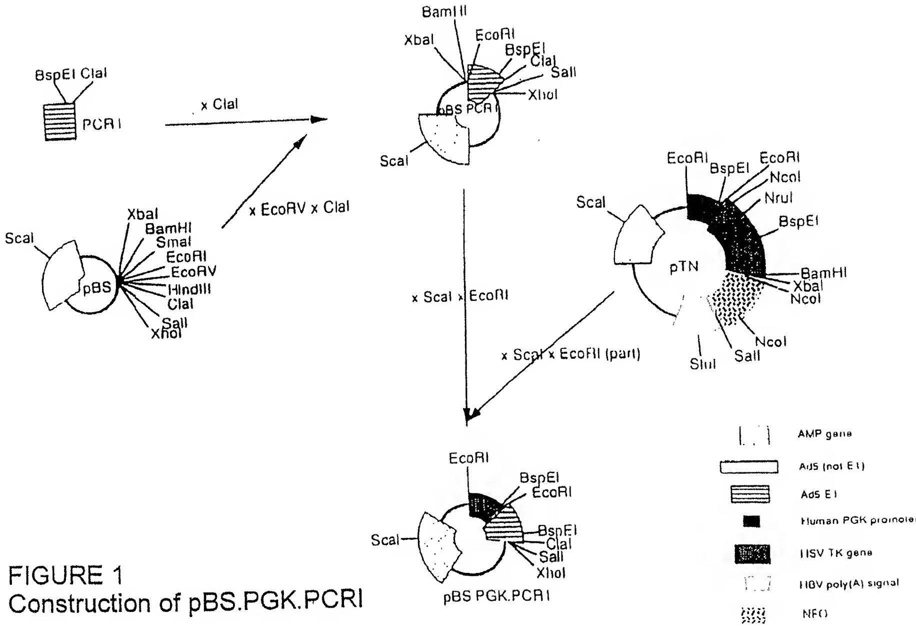


FIGURE 1
Construction of pBS.PGK.PCRI

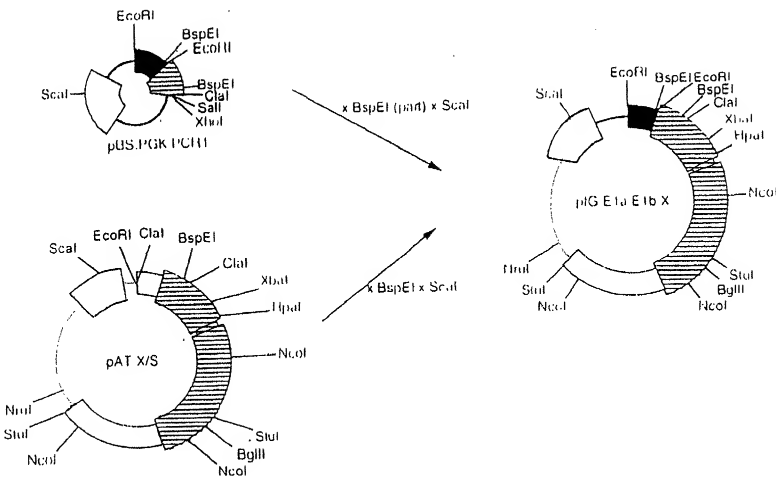


FIGURE 2
Construction of pIG.E1a.E1b.X

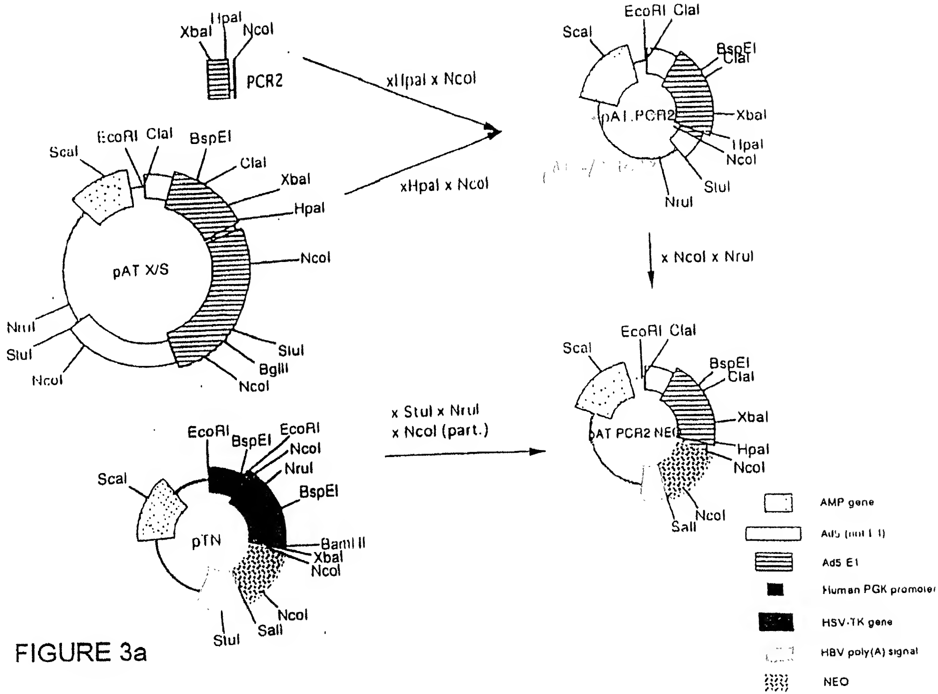

Xbal
Hpal
Ncol
PCR2
xHpal x Ncol
Scal
EcoRI Clal
BspEI
Clal
Xbal
Hpal
Ncol
pAT X/S
Nrul
Stul
Ncol
Stul
BglII
Ncol
xHpal x Ncol
pAT.PCR2
Xbal
Hpal
Ncol
Stul
Nrul
x Ncol x Nrul
x Stul x Nrul
x Ncol (part.)
EcoRI
BspEI
EcoRI
Ncol
Nrul
BspEI
BamHI
Xbal
Ncol
pTN
Ncol
Sall
Stul
pAT PCR2 NEO
Sall
AMP gene
Ad5 E1
Human PGK promoter
HSV-TK gene
HBV poly(A) signal
NEO

FIGURE 3a

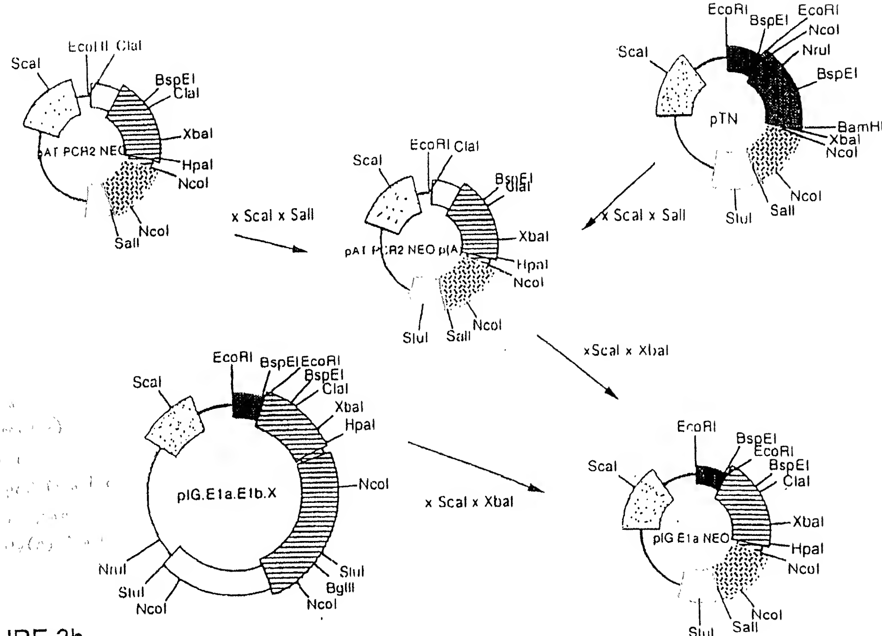


FIGURE 3b
Construction of pIG.E1a.NEO

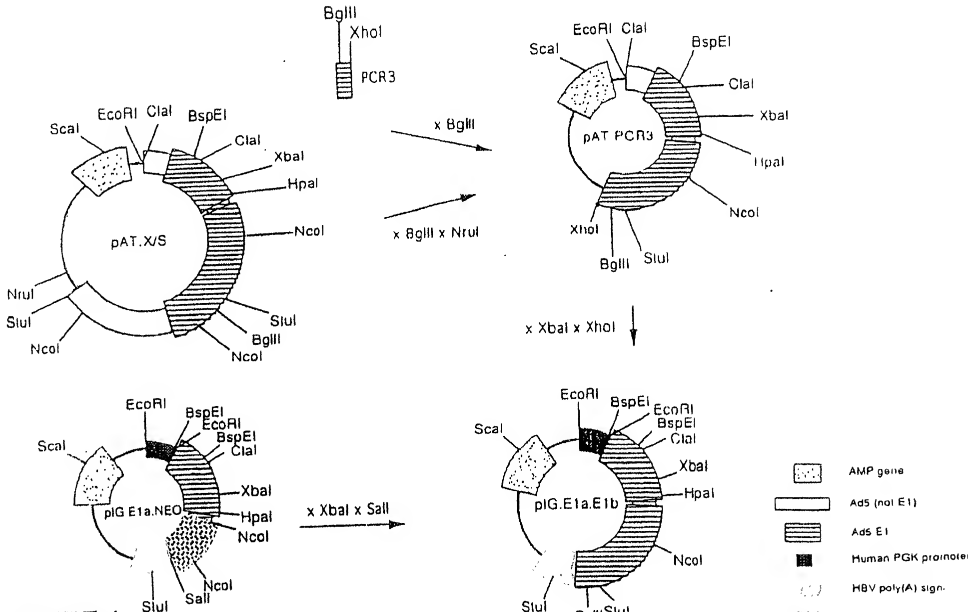


FIGURE 4
Construction of pIG.E1a.E1b

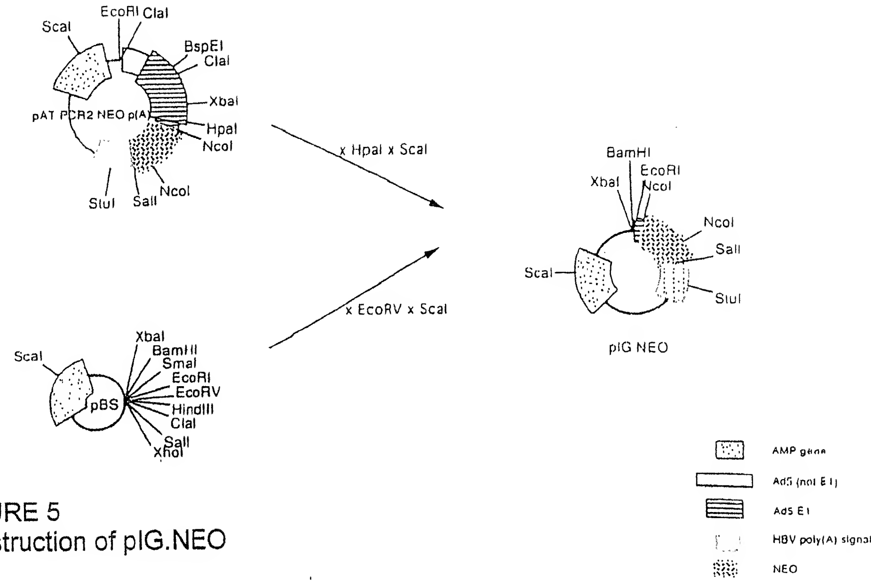
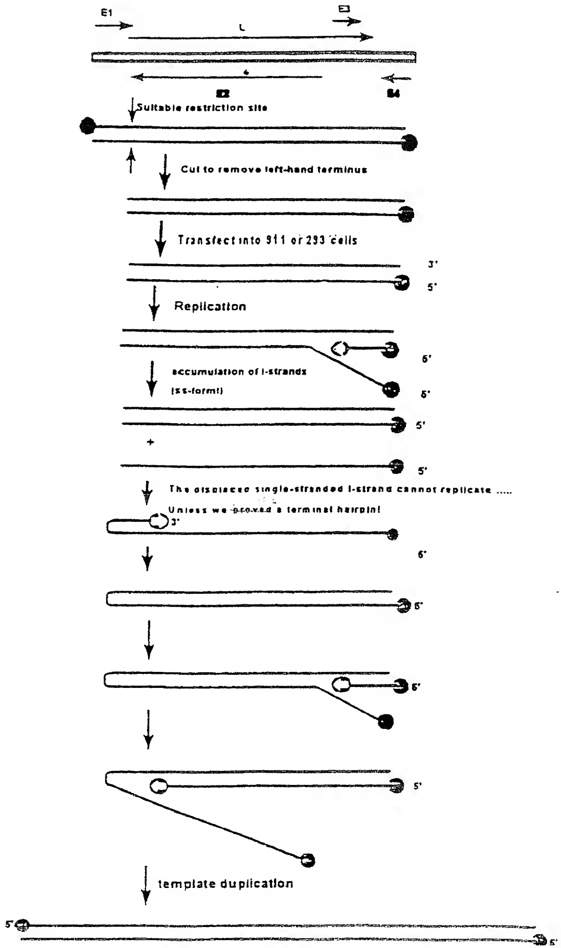


FIGURE 5
Construction of pIG.NEO

FIGURE 13


E1
L
Suitable restriction site
Cut to remove left-hand terminus
Transfect into 911 or 293 cells
3'
5'
Replication
accumulation of l-strands
(ss-form!)
+
The displaced single-stranded l-strand cannot replicate .....
Unless we provide a terminal hairpin!
template duplication